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Impact of extended total time off feed on lamb eating quality

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Abstract

MSA ™ guidelines for lamb producers and processors ensure a consistent and predictable quality of lamb meat is produced. However, some prime lamb producers are located at distances or situations that result in feed curfew periods falling outside of the guidelines. This project was undertaken to investigate alternative pathways for the supply of lamb to MSA specifications and to examine whether there are any negative welfare implications of these alternative pathways.

Lambs from Kangaroo Island (KI) and from within 2.5h travelling time of the processor had objective measurements of the loin (fresh colour, ph-temperature window, ultimate pH) consistently within threshold limits to achieve acceptable eating quality. There was no difference in shear force (tenderness) of the loin or topside between lamb source. There was no adverse effect of feed curfew (48h, 76h, 91h) on carcase weight or objective eating quality measurements of the loin. There was a significant interaction between lamb source and curfew on ultimate pH of the leg, with lambs with longer curfews having lower values. Lambs from KI were more likely to have dark cutting loins and legs.

None of the welfare indicators (plasma osmolality, urine specific gravity, liveweight loss in lairage, muscle glycogen) were outside normal ranges for healthy sheep, with evidence to suggest that sheep accessed water in lairage.

Extending the feed curfew time of lambs from 48h up to 90h will not detrimentally affect objective measures of eating quality of the loin or affect key animal welfare indicators.

Executive Summary

Meat Standards Australia™ (MSA) has developed guidelines to ensure a consistent and predictable quality of lamb meat is produced; one of the guidelines being that the time off feed from mustering to slaughter is between 12 and 48h (MSA-TTS1). However significant numbers of prime lamb producers are located at distances from processing plants that result in on-farm curfew, travelling and lairage time that exceeds 48 hours, often up to 72h. . This project was undertaken to investigate alternative pathways for the supply of lamb to MSA specifications and to examine whether there are any negative welfare implications of these alternative pathways.

In total, 1540 lambs were killed over three non-consecutive weeks from a total of six producers from each of two geographical regions of South Australia – Kangaroo Island (KI) and properties not more than 120km from the processing plant (Near). There was no effect of source or curfew time (48-51h, 72h, or 90h) on carcase weight or fat score, however, carcase weight tended to decrease with increased time in lairage, with an average 400g loss between 48 and 90h in lairage. While statistically insignificant, this loss may have repercussions from a processors point of view.

Source and lairage time had little effect on the objective measures of eating quality of the loin, with no change in loin tenderness, pH decline, ultimate pH or the proportion of samples with pH > 6.0. The ultimate pH of the loin was consistently under the threshold value of 5.8, above which eating quality can be affected. Loin glycogen was higher in Near lambs, however, within source there was no change with time in lairage, and values were consistently above the threshold of 0.8g/100g tissue, below which eating quality can be affected. An interaction between source and curfew time in the ultimate pH of both leg muscles did not translate into any differences in the shear force of the topside, aside from a significant decrease in shear force at 90h. There was no effect of lairage time on the proportion of carcasses with ultimate pH > 6.0 (dark cutting carcasses), however significantly more lambs from KI had dark cutting legs and loins.

The two key colour traits, lightness and redness were not affected by increasing curfew period, with the exception that redness increased with 72h curfew. There was a significant increase in *b** (yellowness) as curfew time increased, particularly in lambs from Near properties, however, yellowness is generally not associated with consumer preference of lamb and is therefore unlikely to have an effect on consumer acceptability (Khliji *et al.*, 2010). Surprisingly, many lambs from both sources and across curfew times failed to reach acceptable levels for *L** (lightness), which consumers associate with quality and freshness, but 100% of lambs achieved acceptable redness.

The impact of travelling from KI and extended lairage on animal welfare was evaluated by measurement of plasma osmolality, urinary specific gravity (USG), muscle dry matter, liveweight loss in lairage and skin cleanliness. There was no interaction between source or time in lairage with USG, dry matter or cooking loss of the loin. Plasma osmolality was highest in Near lambs at 48h curfew, and decreased with time in lairage, but remained unchanged for KI lambs with time in lairage. In contrast, cooking loss of the SM was highest for Near lambs at 48h curfew, indicating a greater level of tissue hydration. When a USG of >1.045 was considered the threshold for dehydration, significantly more lambs from KI were classified as dehydrated, regardless of time in lairage. Live weight of KI lambs tended to increase with time in lairage, most likely as a result of rehydration, while the live weight of Near lambs fluctuated

Overall, there was little effect of increasing time in lairage from 48 to 90h on the objective measures of eating quality, or the animal welfare indicators measured here. The increased incidence of dehydration and dark cutting carcasses, and the decreased levels of glycogen in lambs from KI compared to Near lambs suggests that they may experience more stress throughout transport, however this did not translate into significantly different meat eating quality.

In conclusion, it is possible that feed curfew period can be extended up to 72h (and even to 90h) as an alternative pathway for the supply of lamb to MSA specifications. It is important to ensure that lambs have access to water to prevent dehydration whilst in lairage. For lambs with extended or complicated transport, such as road and ferry, it will be necessary to ensure that lambs have excellent on-farm nutrition to ensure adequate muscle glycogen to buffer against the stress imposed by extended transport, and decrease the risk of dark cutting meat.

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Background

Prolonged time off feed and water prior to slaughter can reduce dressing percentages (Jacob *et al.*, 2005a), compromise animal welfare (Hogan *et al.*, 2007), and may alter the eating quality of lamb meat. Therefore, many producers endeavour to minimise the curfew length between on-farm yarding and slaughter. Conversely, many transport companies and processors require animals to be off feed for a minimum of 12 hours prior to being transported or presented for slaughter to aid in cleanliness, microbiological control and food safety (Gregory *et al.*, 2000). As such, a compromise between minimising carcase weight loss and the processor requirements for clean stock is necessary.

Meat Standards Australia™ (MSA) has developed guidelines to ensure a consistent and predictable quality of lamb meat is produced; one of the guidelines being that the time off feed from mustering to slaughter is between 12 and 48h (MSA-TTS1). However significant numbers of prime lamb producers are located at distances from processing plants that result in on-farm curfew, travelling and lairage time that exceeds 48 hours, often up to 72h. For example, lambs from Kangaroo Island require 24h feed curfew prior to travelling on the ferry which adds significantly to the curfew period.

While it is not known whether increasing time off feed prior to slaughter will affect objective measures of lamb eating quality, there was no effect of total curfew time ranging from 15.6 to 91h on subjective consumer scores for smell, tenderness, juiciness or liking of flavour of the loin for lambs (Jacob *et al.*, 2005a). In Merino wethers, there was no effect of increasing post-transport lairage time from 24h to 72h on muscle dry matter, colour or LL shear force, although cooking loss decreased with time in lairage (Toohey *et* al., 2006). Likewise, glycogen concentration and pHu of leg muscles within consignments were unaffected by total curfew time (from 15.1 to 96.25h), although glycogen concentration tended to increase with time in lairage post-transport (Jacob *et al.*, 2005b). Indeed, several authors have hypothesised that increased time in lairage with access to water may allow lambs to replenish muscle glycogen levels, reduce tissue dehydration and carcase weight loss after the acute stress of transport (Liu *et al.*, 2012), however the optimal time is not known.

The welfare concerns that arise with increasing time off feed prior to slaughter are primarily related to dehydration. During time in lairage, lambs can fail to drink due to the unfamiliar environment, and the incidence of sub-clinical dehydration in lambs in some Australian abattoirs is as high as 60% (Jacob *et al.*, 2006a). Urine specific gravity (USG) is a measure of recent water intake and tissue hydration, and is affected by age, month of kill and farm curfew time but unaffected by total transport-lairage time in 219 commercial consignments in WA (Jacob *et al.*, 2006a).

Current animal welfare standards and guidelines and those under consideration specify that sheep greater than 4 months old must not be deprived of water for greater than 48h. Feed deprivation exceeding 48 hours should be avoided, except where reasonable management practices such as preparation for sale, transport, slaughter and drenching result in a longer period of deprivation (Australian Animal Welfare Guidelines-Sheep, Public consultation version 1.0). Prior to slaughter, resting periods of up to 96h post-transport with access to water are recommended (Guidelines for Slaughtering Establishments CSIRO 2001)

This project aims to determine whether extending the total time from mustering to slaughter from 48 to 72h has an impact on objective measures of lamb eating quality and animal welfare indicators.

Project objectives

1. Evaluate the effect of total time off feed pre-slaughter of not greater than 90h on animal welfare status indicators such as liveweight change, urine specific gravity, muscle dehydration and blood osmolality.
2. Investigate alternative pathways for the supply of lamb to MSA specifications as measured by ultimate pH, pH decline, meat colour and shear force (day 5).

Methodology

Trial Design

Lambs (N=1540) were slaughtered in the weeks commencing 22nd October, 5th November and 3rd December, 2012, from a total of twelve different suppliers; six from each of two geographic regions in South Australia – Kangaroo Island (KI – Parndana and Kingscote) and from within 120km of the processing plant (Near – Lyndoch, Greenock, Tarlee, Saddleworth, Torrens Vale and Yankalilla). Due to time frame and logistics, it was not possible to source lambs from the same suppliers for the different weeks, as initially planned.

Lambs were all crossbred lambs with a terminal sire. Dam breed or lamb age was not provided by the producers, but was likely to range from four to seven months of age. All lambs were weaned and finished on green pasture, nearing senescence.

The trial was conducted over three non-consecutive weeks, with lambs being killed from two producers from each region for each of the three weeks (a total of four producers per week; Table 1). Near lambs were killed on the Monday, Tuesday and Wednesday of each week, while KI lambs were killed on Tuesday and Wednesday only.

Table 1. Source and number of lambs in each kill group for each of the three trial weeks.

	Farm code	Monday	Tuesday	Wednesday	Total
Week 1	Near 1	50	53	49	152
	Near 2	50	54	55	159
	KI 1	-	60	62	122
	KI 2	-	48	54	102
Week 2	Near 3	50	50	51	151
	Near 4	45	45	40	130
	KI 3	-	50	50	100
	KI 4	-	49	50	99
Week 3	Near 5	45	43	45	133
	Near 6	49	51	50	150
	KI 5	-	61	59	120
	KI 6	-	61	61	122
Total		289	625	626	1540

Near lambs met MSA requirements (less than 48h curfew between on-farm mustering and slaughter) and KI lambs were slaughtered 51h after on-farm mustering (Table 2). These lambs were classed as 48h for data analysis. Feed curfew significantly outside of MSA curfew requirements were classed as 72h curfew and 90h curfew (Table 2).

Table 2. Total time off feed curfew (yarding to slaughter), and the curfew class allocation.

Class	**48h class**		**72h class**		**90h class**	
Farm	Near	KI	Near	KI	Near	KI
1	46 h	51 h	70 h	75 h	94 h	-
2	45 h	51 h	69 h	75 h	93 h	-
3	46 h	51 h	70 h	75 h	94 h	-
4	46 h	51 h	70 h	75 h	94 h	-
5	38 h	51 h	62 h	75 h	86 h	-
6	38 h	51 h	62 h	75 h	86 h	-

There was greater variation in on-farm yarding times than was originally anticipated, with four consignments of Near lambs yarded on Saturday morning, and one on Saturday evening, rather than on Sunday morning as was planned. Therefore, all except two Near farms had a 24h pre-transport curfew, rather than the 6h pre-transport curfew required to kill lambs with a total curfew period of 24h. Average travel time was just over 2hrs for the Near lambs and between 6 and 11h for the KI lambs (Table 3), resulting in an average difference in water curfew period of 10h between the two lamb sources. Time in lairage was similar across lamb source (Table 3).

Table 3. On-farm feed and water curfew, approximate travel time and time in lairage prior to each kill.

Source	**On-farm curfew (h)**	**Travel time (h)**	**Total time off water (h)**	**Time in lairage (h)**		
				First kill	Second kill	Third kill
Near 1	24	2	26	17	41	65
Near 2	24	2.2	26.2	17	41	65
Near 3				20	44	68
Near 4	24	2.5	26.5	20	44	68
Near 5	14	2.3	16.3	22	46	70
Near 6	14	2.5	16.5	22	46	70
Average		2.3	23	20	44	68
KI 1	24	6	30	21	45	
KI 2		6		21	45	
KI 3	24	11	35	16	40	
KI 4	24	11	35	16	40	
KI 5	24	8	32	19	43	
KI 6	24	8	32	19	43	
Average		8.3	33	19	43	

Measurements and sample collection

After un-loading from the truck, animals were weighed in lairage using commercial sheep scales and had skin cleanliness scores recorded (Warner *et al*., 2012). Lambs were then split into kill groups immediately after weighing, with the first 50 allocated to the 48h kill group, the second 50 allocated to the 72h kill group etc. They were re-weighed approximately 2 hours prior to slaughter, and had another skin cleanliness score assessment.

Eating quality traits

pH decline was measured in 1460 carcases in the left, caudal end of the *m. longissimus thoracic et lumborum* (LL), as soon as the carcasses entered the chiller, and when carcases reached 18°C and 12°C. At approximately 20h post-mortem, pH levels were recorded the left LL at the 13th rib and in the *m. semitendinosus* (ST) and *m. semimembranosus* (SM) as an estimate of ultimate pH.

Within 5h of slaughter, a 2g core sample of LL was collected from 298 randomly selected carcases above the 13th rib on the left side, placed into a tube and immediately placed into liquid nitrogen (N_2) for storage and subsequent analysis for glycogen concentration. Samples were homogenised (30mM HCl) and then analysed for lactate and glucose using an Olympus AU 400 autoanalyser; with an enzymatic kit for lactate and a glucose kit (Cat.No OSR6121) for glucose. Total glycogen was calculated by halving the lactate value and adding it to the glucose value.

Fresh eye muscle colour was measured on 196 carcases approximately 22 hours post-mortem on a section of LL that had been cut in a transverse direction between the 12th and 13th ribs, and allowed to 'bloom' for 30-60 minutes. A Minolta Chromameter was used to measure lightness (L^*), redness (a^*) and yellowness (b^*); and Chroma and hue were calculated (Chroma, $C^* = (a^{*2}+b^{*2})^{0.5}$; hue, $h = \tan^{-1}[b^*/a^*]$ (Jacob *et al.*, 2006a).

At approximately 26h post-mortem, the topside and the left section of the LL were removed from the carcase. Samples (65g) from both muscles were vacuum packed and aged (4-5ºC) for five days prior to freezing at -20ºC. The samples were weighed before and after cooking to determine cooking loss. These samples were processed and measured using a Lloyd instrument to determine shear force (Hopkins and Thompson, 2001). Shear force results are presented using the SI unit of force - Newtons (N) - rather than the non SI unit - kgF. Shear force can be converted to kgF by the following equation:

$$1 \text{ kgF} = 0.109716 \text{ N}.$$

Welfare indicators

Blood (5 - 10ml) was collected from the jugular wound of 575 lambs at the point of exsanguination into K_3EDTA vacutainers. Blood was centrifuged within 3h of collection and plasma was frozen and stored at -20^{o}C. Plasma osmolality was measured using an Advanced® Model 3MOplus 20µl micro-osmometer.

Bladders from 543 randomly selected lambs were collected from the evisceration table on the slaughter floor. Urine (3-4ml) was extracted from the bladders within 5h, frozen and stored at -20^{o}C. Urine specific gravity (USG) was measured using a RHC-200/ATC Clinical Veterinary Refractometer. A benchmark USG of 1.045 was used to classify a lamb as being dehydrated, as this is considered the upper normal range of USG for sheep, and a point at which tissue hydration should be affected (Jacob *et al.*, 2006a).

Approximately 10g of loin muscle was collected 26h post-mortem for dry matter analysis. Samples were frozen immediately after collection, weighed and then placed frozen into an industrial oven for 3 days at 72ºC. The samples were then weighed every 3 hours until there was no change in weight with further drying, and then DM expressed as a percentage.

Statistical Analysis

Data was analysed to detect whether differences existed between Near and KI lambs within the 48h 'MSA' class (ie is there an effect of a 51h curfew and/or ferry trip compared to lambs that have less than 48h curfew and only travel by road) and whether there was a difference between 48h, 72h and 90h curfew.

Continuous data was analysed using mixed models (REML; SAS v9.3). Source (KI, Near) and Curfew (MSA; 72h, 90h) and their interaction were included as fixed effects and Site (KI 1-6; Near 1-6), Week (1, 2, 3) and Slaughter Day (Mon, Tues, Wed) were included as random effects in all models. Temperature and pH meter were included as covariate and random effect in the analysis of the pH data. The following results are the LSM generated from these models.

Results

Liveweight and carcase weight

On-farm live weight at dispatch was not recorded as the participating producers did not have access to scales on property. The unadjusted mean liveweight of the lambs from each of the properties at arrival at lairage and immediately prior to slaughter are shown in Table 4. Due to problems with scales in lairage, liveweights of Near 1 and 2 lambs with 72h curfew were 8kg lighter than the unload liveweights and the 48h and 90h pre-slaughter liveweights and the unload liveweights of the Near 3 and Near 4 lambs were 4-6kg lighter than the 48h and 72h pre-slaughter liveweights, so these were removed from the analysis, as this liveweight change is unlikely to be due to rehydration. The final number of liveweights, carcase weights and palpated fat thickness used for the analysis and the raw means and their ranges are presented in Table 5.

Table 4. Unadjusted mean liveweight of each of the properties immediately after unloading at lairage and two hours prior to slaughter after approximately 48, 72 and 90 hours total curfew period.

Property	Unload	48h	72h	90h
KI 1	42.8	41.4	41.9	
KI 2	40.5	39.3	44.4	
KI 3	45.0	45.7	49.7	
KI 4	46.3	48.0	50.6	
KI 5	41.8	44.0	42.9	
KI 6	48.1	52.0	46.2	
Near 1	51.9	50.0	*42.9*	48.1
Near 2	42.0	43.1	*33.4*	39.3
Near 3	*43.4*	47.4	50.0	46.9
Near 4	*43.2*	47.4	49.3	46.6
Near 5	48.5	46.9	48.3	47.6
Near 6	47.2	46.0	48.1	53.0

Table 5: Descriptive statistics of lamb liveweight, carcase weight and palpated works fat.

Measurement	Number	Mean ± s.d.	Minimum	Maximum
Unload weight (kg)	1223	45.6 ± 6.62	31.2	76.6
Pre-slaughter weight (kg)	1431	46.5 ± 6.06	30.2	69.2
Hot carcase weight (kg)	1540	22.6 ± 3.03	15.5	34.7
Fat (mm)	1540	15 ± 4.6	5	25

There was no difference in liveweight between KI and Near lambs (P=0.13) but there was a significant effect of time on liveweight (P<0.001) and a significant interaction between lamb origin and curfew time (P<0.0001; Figure 1a). KI lambs were significantly lighter on arrival at lairage compared to pre-slaughter liveweights, whereas Near lambs were heavier at arrival at lairage compared to their pre-slaughter liveweights after 48 and 90 hours curfew.

There was no difference in carcase weights of lambs from the different locations (P=0.8). There was a small, but non-significant (P=0.08) decrease in carcase weights of lambs slaughtered as curfew time increased (Figure 1b), with the 90h curfew lambs having carcase weights 0.4kg less than the lambs that had 48h feed curfew (P=0.03). There was no interaction between source of lamb and curfew time. There was no effect of lamb source or curfew time on palpated fat when HCWT was included as a covariate.

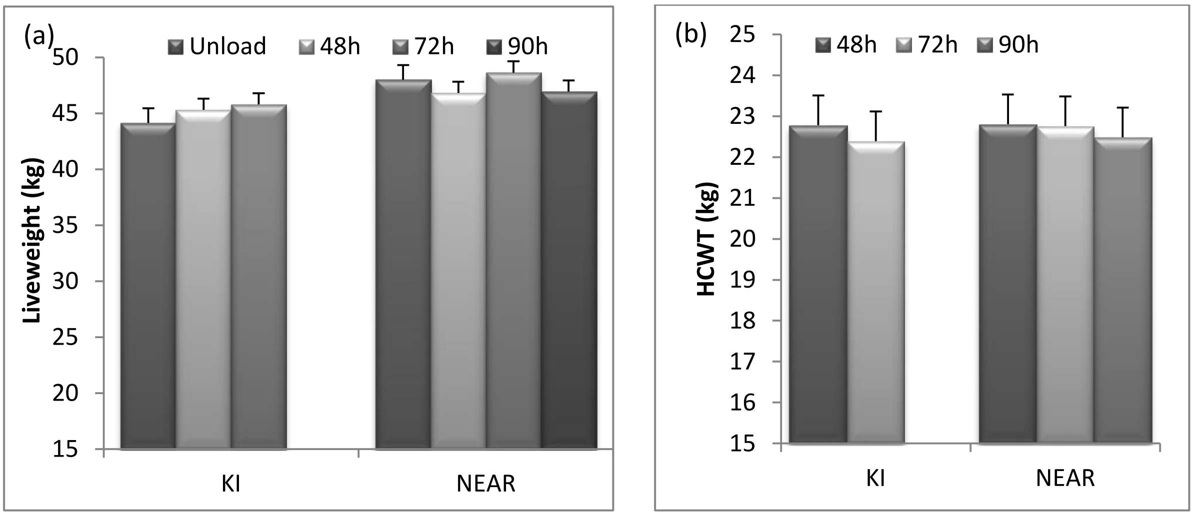


Figure 1. Mixed model (a) liveweight and (b) carcase weight means and approximate standard errors immediately after unloading and after 48, 72 or 90 hours feed curfew.

Eating quality

The number of samples measured for objective eating quality, the raw means, standard deviations and ranges of values are shown in Table 6. All values are within normal ranges, with the exception the average shear force were higher than expected across the trial, although the range for the LL is similar to that in the INF (11 - 95N; Mortimer *et al.*, 2013). One possible reason for the higher SF values is that the Lloyd was due for calibration, so the absolute values may be inaccurate, however the contrasts between treatments is still assessable.

Table 6: Descriptive statistics of objective eating quality measurements.

Measurement	Number	Muscle	Mean ± s.d.	Minimum	Maximum
Apparent pHu LL	1413	LL	5.74 ± 0.20	5.10	6.83
Apparent pHu SM	896	SM	5.63 ± 0.13	5.32	6.21
Apparent pHu ST	1415	ST	5.91 ± 0.24	5.35	6.87
Shear force at day 5 (N)	300	LL	47.1 ± 14.64	27.4	112.9
Shear force at day 5 (N)	300	SM	99.4 ± 23.97	37.0	164.3
Cooking loss (%)	296	LL	26.0± 3.74	12.6	38.5
Cooking loss (%)	297	SM	24.9 ± 2.67	11.3	33.0
Lightness (L*)	196	LL	32.4 ± 2.57	26.7	39.2
Redness (a*)	196	LL	21.2 ± 1.76	14.8	25.4
Yellowness (b*)	196	LL	2.6 ± 1.67	-2.0	7.6
Chroma (C*)	196	LL	21.4 ± 1.89	14.9	26.1
Hue (h)	196	LL	6.8 ± 4.17	-6.2	17.9

There was no difference between lamb source or curfew time in the pH of the loin at 18^{o}C, but there was a significant interaction (P=0.022; Table 7). Lambs from KI had a higher pH at 18^{o}C after 72h curfew compared to 48h curfew, however there was no effect of curfew time on pH at 18^{o}C in the Near lambs. Neither lamb source nor curfew time had an effect on loin temperature at pH 6.0 (Table 7). Despite acceptable pH at 18 degrees and temperatures at pH 6.0, under MSA recommended processes, 39% of lambs from Near locations and 27% of lambs from KI did not pass through the pH-temp window of less than ph6 between 18 to 35^{o}C. However 57% of these lambs did not pass through the pH-temp window because they were below pH 6 at the first pH measurement on the hot carcase. As heat shortening does not tend to be a problem in lamb, if the upper limit of 35^{o}C is removed, approximately 20% of lambs were at risk of cold shortening (Table 7). This level is what is generally seen within commercial plants. Nevertheless, there was no effect of lamb source or curfew time on the number of lambs that passed through the pH-temp window. A proportion of carcases did not pass through the window because they were dark cutters (Table 9).

Table 7. Loin pH at 18 degrees (pH18), loin temperature at pH 6.0 (pH6Temp) and percentage of lambs that passed through the pH temp window (ph<6.0 at >18^{o}C). Model and random effects for each of the traits are shown.

	pH18			**pH6Temp**			**ph<6.0 at >18^{o}C**		
	48h	**72h**	**90h**	**48h**	**72h**	**90h**	**48h**	**72h**	**90h**
KI	5.77^a	5.83^b		26.8	26.4		86%	79%	
NEAR	5.82^{ab}	5.79^{ab}	5.83^{ab}	25.6	27.3	26.2	78%	84%	77%
P=	ns	ns		ns	ns				
Model	source | curfew								
Random	Site Meter SLDay(Week)								

There was no difference between KI and Near lambs or curfew times in ultimate pH measures of the loin and there was no interaction between lamb source and curfew period (Table 8). However, pHST was significantly higher in the KI lambs (P=0.031).and there was a significant interaction between lamb source and curfew time (P=0.050). KI lambs with a 48h curfew had a significantly higher pHST than KI lambs with 72h curfew (P=0.04), but there was no effect of curfew time on pHST within the Near lambs (Table 8). In contrast, there was no effect of lamb source on pHSM, but there was a significant effect of curfew (P=0.002) and a significant interaction (P=0.004). Lambs with a 48h curfew had a higher pHSM than lambs with a 72h curfew (P=0.001). Likewise, Near lambs had a lower pHSM after 90h curfew compared to Near lambs with a 72h (P=0.001) or 48h curfew (P=0.01).

Table 8. Ultimate pH of the loin (pHLL), the *semitendinosis* muscle (phST) and the topside (pHSM). Model and random effects for each of the traits are shown.

<table>
<tr><th></th><th colspan="3">pHLL</th><th colspan="3">pHST</th><th colspan="3">pHSM</th></tr>
<tr><th></th><th>48h</th><th>72h</th><th>90h</th><th>48h</th><th>72h</th><th>90h</th><th>48h</th><th>72h</th><th>90h</th></tr>
<tr><td>KI</td><td>5.69</td><td>5.71</td><td></td><td>6.06^{a}</td><td>5.94^{b}</td><td></td><td>5.71^{a}</td><td>5.54^{bc}</td><td></td></tr>
<tr><td>NEAR</td><td>5.63</td><td>5.64</td><td>5.64</td><td>5.84^{bc}</td><td>5.91^{bc}</td><td>5.82^{c}</td><td>5.63^{a}</td><td>5.66^{ab}</td><td>5.53^{c}</td></tr>
<tr><td>P=</td><td>ns</td><td>ns</td><td></td><td>0.0035</td><td>ns</td><td></td><td>0.06</td><td>0.008</td><td></td></tr>
<tr><td>Model</td><td colspan="6">TEMP source | curfew</td><td colspan="3">source| curfew</td></tr>
<tr><td>Random</td><td colspan="3">Site Meter SLDay(Week)</td><td colspan="6">Site SLDay(Week)</td></tr>
</table>

There was no effect of curfew time on the proportion of dark cutting carcases (Table 9), however there was a trend (P=0.07) for there to be more dark cutting loins and legs (ST; P<0.001) in the KI lambs than in the Near lambs. The relative risk of KI lambs having pHu > 6.0 of the ST was 2.16 (CL=1.38-3.37) and of the loin was also 2.16 (CL=0.93-5.02).

Table 9. Proportion of lambs with pHu > 6.0 in the loin, ST and SM.

	Loin			**ST**			**SM**		
	48h	**72h**	**90h**	**48h**	**72h**	**90h**	**48h**	**72h**	**90h**
KI	16%	16%		50%	38%		1%	1%	
NEAR	6%	9%	9%	16%	25%	21%	0	1%	0

Overall the Near lambs had 0.119g glycogen/100g tissue more than the KI lambs (P=0.04). There was no significant difference in shear force of the loin across lamb origin or curfew time. Shear force of the topside was significantly lower (P>0.001) after 90h curfew, but there was no effect of lamb source (Table 10). There were no interactions between lamb source and curfew period for glycogen content or shear force after five days aging. With increasing curfew period, approximately 10% more samples did not meet consumer acceptability (Table 10) based on a cut off of 40N (Hopkins *et al.*, 2006).

Table 10. Glycogen content of loin and shear force after 5 days aging of loin and topside. Proportion of loin considered acceptable shown in parentheses (SF<40N). Model and random effects for each of the traits are shown.

	Glycogen (g/100g tissue)			**Loin (N)**			**Topside (N)**		
	48h	**72h**	**90h**	**48h**	**72h**	**90h**	**48h**	**72h**	**90h**
KI	0.96	0.94		47.4 (52%)	45.3 (45%)		103.5	100.4	
NEAR	1.06	1.07	1.06	49.2 (60%)	46.3 (50%)	47.3 (37%)	101.9	101.7	89.3
Model	Source Curfew								
Random	Site SLDay(Week)			Site Cook SLDay(Week)			Site Week		

There was no effect of source of lambs or curfew period on meat lightness (L^*; Table 11). MSA lambs were less red than lambs with a 72 (P=0.03) or 90h curfew (P>0.001; Table 11) and there was no effect of lamb source on redness (a^*). There was a significant increase in b^* (yellowness) with increased curfew time (P=0.006) and there was a significant interaction between lamb source and curfew time (P=0.012). The Near lambs with 48h curfew were less yellow than the Near lambs with 72 or 90h curfew (Table 11). All lambs achieved acceptable redness (a^* > 14) but less than 36% of the lambs had acceptable lightness (L^* > 34; Table 11). This was consistent across source of lamb and curfew period. Neither week, nor day were significant effects so were not included in the models for colour analysis.

Table 11. Colour and acceptability of lamb carcases.

	L*			**L acceptable**			**a***			**b***		
	48h	**72h**	**90h**	**48h**	**72h**	**90h**	**48h**	**72h**	**90h**	**48h**	**72h**	**90h**
KI	31.9	32.9		10%	32%		20.8	21.4		2.8^{ab}	2.9^{a}	
NEAR	33.2	33.4	32.8	30%	5%	36%	20.4	21.2	21.7	1.7^{b}	3.2^{a}	2.7^{a}

Chroma of lamb loins was not affected by lamb source, however 48h curfew lambs had a lower chroma than the longer curfew lambs (P=0.0009; Table 12). There was no effect of source of lamb on hue, however 48h curfew lambs had a lower hue (P=0.005) than 72 or 90h curfew lambs. There was also a significant interaction between lamb source and curfew (P=0.0036), as Near lambs with a 48h curfew had a lower hue angle than all other lambs (Table 12).

Table 12. The chroma and hue of lamb carcasses.

	Chroma			**Hue**		
	48h	**72h**	**90h**	**48h**	**72h**	**90h**
KI	20.8	21.4		7.47^{a}	7.52^{a}	
NEAR	20.4	21.2	21.7	4.25^{b}	8.62^{a}	6.97^{ab}

Welfare indicators

The impact of travelling from KI and extended lairage on animal welfare was evaluated by measurement of plasma osmolality, urinary specific gravity (USG) and muscle dry matter. These measurements are used to assess dehydration of the lambs (Jacob *et al.*, 2006a). The number of samples measured as indicators of animal welfare, the raw means, standard deviations and ranges of values are shown in Table 13.

Table 13. Descriptive statistics of animal welfare indicators.

Measurement	**Number**	**Muscle**	**Mean ± s.d.**	**Minimum**	**Maximum**
Urine specific gravity	543	n.a.	1.034 ± 0.0101	1.005	1.060
Osmolality (mOsm/L)	594	n.a.	323 ± 14.6	300	472
Dry muscle weight (%)	299	LL	25.6 ± 1.32	22.3	29.3
Glycogen (mg/100g)	299	LL	1.018 ± 0.2152	0.404	1.589

Overall, there was no effect of lamb source on plasma osmolality or average USG (Table 14). Significantly more lambs from KI (P<0.005) were classed as dehydrated (USG > 1.045) when USG was used as an indicator. Conversely plasma osmolality was higher for Near lambs slaughtered at MSA recommended times (P<0.05) and the MSA lambs had higher plasma osmolality than the lambs with 72h and 90h curfew (P<0.001).

Table 14. Plasma osmolality (mOsmol/L), urinary specific gravity (USG) and the proportion of lambs classified as dehydrated (USG > 1.045).

	Plasma osmolality			**USG**			**Dehydrated lambs**		
	48h	72h	90h	48h	72h	90h	48h	72h	90h
KI	321.9[a]	320.8[a]		1.0362	1.0361		28%[a]	28%[a]	
NEAR	325.4[b]	320.5[a]	320.1[a]	1.0325	1.0336	1.0334	12%[b]	7%[b]	13%[b]

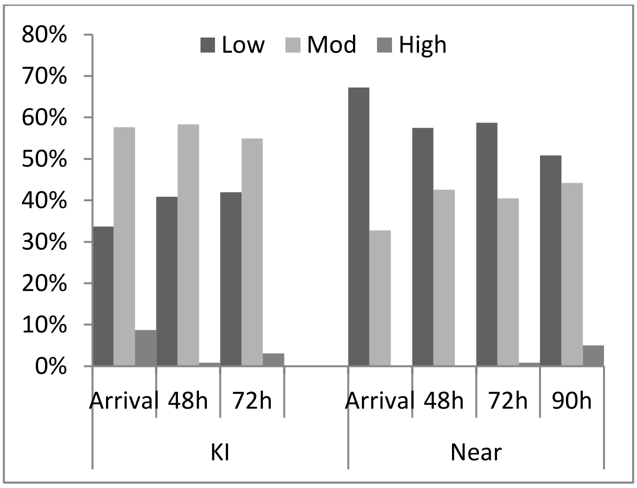
Near lambs had a significantly higher cooking loss from the loin (P=0.03) than the KI lambs but there was no effect of curfew time on cooking loss of the loin and no interaction (Table 15). The topside followed similar trends but the differences were not significant. There was no effect of lamb source or curfew on muscle dry matter.

Table 15. Cooking loss of the loin and topside and dry matter (DM) of the loin.

	Loin (%)			**Topside (%)**			**Loin DM (%)**		
	48h	**72h**	**90h**	**48h**	**72h**	**90h**	**48h**	**72h**	**90h**
KI	24.8[a]	25.2[a]		24.7	24.3		25.8	25.6	
NEAR	27.7[b]	25.9[ab]	25.0[a]	26.3	24.7	24.4	25.4	25.6	25.9

There was a significant effect of both lamb source (P=0.013) and curfew time (P=0.021) on skin cleanliness score and a significant interaction (P=0.001; Figure 2.). Lambs from KI had significantly higher skin cleanliness scores on arrival in lairage than all other lambs and times. Over time, skin cleanliness score decreased in the KI lambs but increased in the Near lambs.

Figure 2. Percentage of lambs with skins likely to have a low (Score 1), medium (Score 2) or high (Score 3) risk of carcase contamination.



Discussion

Overall, increasing feed curfew from 48h to 52h is unlikely to have major negative effects on eating quality of lamb or on the welfare of the lamb (Table 16). In the majority of measurements there was no effect of increasing feed curfew period and for some traits, there was a positive effect.

Of concern, however, is the potential negative effect of lamb source on dark cutting meat. Lambs from KI consistently had less glycogen in their loins and more loins and legs with pH greater than 6.0. However, high apparent pHu in ST is unlikely to be very dark – in loin and ST it's a different matter. Further analysis of colour (L*) and pH>6.0 is required.

Table 16. Summary of effects of increasing feed curfew and the effect of lamb source (lambs from KI compared to Near lambs) on liveweight, objective measures of eating quality and animal welfare indicators. *0=no effect, -ve=Negative effect; +ve=Positive effect.*

<table>
<tr><th></th><th>Small increase in feed curfew 48h to 52h</th><th>Increase feed curfew to 76h</th><th>Increase feed curfew to 91h</th><th>Effect of lamb source</th></tr>
<tr><td></td><td colspan="4">Weight</td></tr>
<tr><td>Live weight</td><td colspan="4">0</td></tr>
<tr><td>HCWT</td><td>0</td><td colspan="2">-ve</td><td>0</td></tr>
<tr><td></td><td colspan="4">Objective measures of eating quality</td></tr>
<tr><td>Lightness (L*)</td><td colspan="4">0</td></tr>
<tr><td>Redness (a*)</td><td>0</td><td>-ve</td><td>0</td><td>0</td></tr>
<tr><td>Yellowness (b*)</td><td>-ve</td><td>-ve</td><td>0</td><td>0</td></tr>
<tr><td>Loin tenderness</td><td colspan="4">0</td></tr>
<tr><td>pH-temp window</td><td colspan="4">0</td></tr>
<tr><td>Loin apperent ultimate pH</td><td colspan="4">0</td></tr>
<tr><td>Loin glycogen</td><td>0</td><td>0</td><td>0</td><td>-ve</td></tr>
<tr><td>Dark cutting loin (pHu > 6.0)</td><td>0</td><td>0</td><td>0</td><td>-ve</td></tr>
<tr><td>Topside tenderness</td><td>0</td><td>0</td><td>+ve</td><td>0</td></tr>
<tr><td>ST apparent ultimate pH</td><td>-ve</td><td>0</td><td>0</td><td>-ve</td></tr>
<tr><td>SM apparent ultimate pH</td><td>-ve</td><td>+ve</td><td>+ve</td><td>0</td></tr>
<tr><td>Dark cutting ST (pHu > 6.0)</td><td>0</td><td>0</td><td>0</td><td>-ve</td></tr>
<tr><td>Dark cutting SM (pHu > 6.0)</td><td colspan="4">0</td></tr>
<tr><td></td><td colspan="4">Welfare indicators</td></tr>
<tr><td>Plasma osmolality</td><td>+ve</td><td>+ve</td><td>0</td><td>0</td></tr>
<tr><td>USG</td><td colspan="4">0</td></tr>
<tr><td>USG > 1.045</td><td>-ve</td><td>0</td><td>0</td><td>-ve</td></tr>
<tr><td>Loin Dry matter</td><td colspan="4">0</td></tr>
<tr><td>Loin cooking loss</td><td>-ve</td><td>0</td><td>0</td><td>+ve</td></tr>
<tr><td>Topside cooking loss</td><td colspan="4">0</td></tr>
</table>

Weight

There was no effect of lamb origin on liveweight or HCWT, and no significant effect of time in lairage on HCWT; which can be used both as an indicator of animal welfare and as a production measure. Lambs from KI consistently increased in weight with time in lairage, while Near lambs tended to fluctuate (Figure 1). Others have also found variable liveweight changes over pre-slaughter fasting periods. Lambs with access to water but fasted for 2 or 4 days prior to slaughter lost 2kg and 1.5kg respectively over the fasting period (Daly *et al*., 2006), whereas lambs gained weight (0.023% of initial liveweight/h) after transport (Thompson *et al*., 1987). They concluded that liveweight losses during transported can be compensated for by an increased intake of water prior to slaughter. Liveweight gain in lairage would suggest that KI lambs were drinking water resulting in re-gaining body weight lost through dehydration during the transport period; however there was not conclusive evidence to support this in the hydration measures taken. This may simply due to increase in gut fill of water and there was not sufficient time for this to affect the blood parameters.

From a lamb producer's viewpoint, there was no effect on HCWT, indicating that it is unlikely that there would significant decrease in producer income. However from a processors view, a 400g decrease in carcase weight over their production cycle, could potentially lead to less meat available for sale. This is only likely to occur if curfews are extended beyond 72h. Others have also found no effect of feed curfew on carcase weight (Daly *et al*., 2006) or small (1.4%-2%) decrease in carcase weight with 72h and 96h feed curfew compared to carcase weights after 48h feed curfew (Thompson *et al*., 1987). This is of a similar magnitude of HCWT loss observed in the current work (1.8% loss at 90h compared to 48h curfew).

Eating Quality

Curfew period had minimal impact on objective measures of eating quality of the loin. Increased curfew period did not affect loin shear force, pH decline, apparent ultimate pH or the proportion of samples with pH > 6.0. Others have also found limited impact of feed curfew on lamb loin pH measurements (Jacob *et* al., 2005b; Daly *et al*., 2006), Merino wether LL shear force (Toohey *et al*., 2006) or lamb loin consumer sensory scores (Jacob *et al*., 2005a). Across both source and curfew time, pHu LL was consistently under the threshold of 5.8, beyond which shelf life and eating quality can be compromised (Hopkins *et al.*, 2007). It is therefore concluded that increasing feed curfew beyond 48h, and up to 90h will not impact loin eating quality.

The pHu of both leg muscles measured was affected by source and time in lairage, whereby lambs from KI that were killed close to the MSA recommendations had significantly higher pHu than the KI lambs killed outside recommendations, and those from Near properties at any curfew length (Table 8). Lambs from KI consistently had a decreasing pHu for both SM and ST with increasing time in lairage, while there was no clear trend for lambs originating from Near properties. All groups had acceptable pHu SM, but high pHu ST (pHu > 5.8); which is expected due to the higher percentage of 2X fast glycolytic fibres in the ST compared with the LL (Hopkins *et al.*, 2007).

High pHu can cause reduced tenderness (Hopkins *et al.*, 2007; Chrystall & Daly, 1996), and the observed differences did translate into higher shear force of the topside compared to the LL, however there were no significant differences between any of the treatments for topside shear force (Table 10). This finding is in agreement with Jacob *et al.* (2005a) who show that despite decreasing pHu with increasing time in lairage, there was overall no difference in subjective consumer scores for smell, tenderness, juiciness and flavour of the lamb.

Within source, there was no change in glycogen concentration with increased time in lairage, in agreement with others who found no effect of 0 to 4d off feed prior to slaughter on muscle glycogen (Jacob *et al.,* 2005a; Daly, *et al*., 2006). This is in contrast to other reports that show glycogen concentrations can increase with time in lairage, as the animal rehydrates and acclimatises to conditions (Liste et al., 2011). The glycogen concentrations across all treatments in this study were above the 0.8g/100g threshold below which poor meat quality can be expected (MSA-TTS5). Muscle glycogen content of 1g/100gm means you are entering the danger zone and likely indicated poor on farm nutrition of the lambs (Pethick pers comm.). It maybe necessary to recommend that lambs from KI need to have additional feeding or growth rates prior to transport to counteract the additional stress involved in ferry travel.

The two key colour traits, lightness and redness were not affected by increasing curfew period, with the exception that redness with 72h curfew. There was a significant increase in *b** (yellowness) as curfew time increased, particularly in lambs from Near properties (Table 11), which is in contrast to other results which show significant decrease in *b** with time in lairage compared to no time in lairage (Liste *et al.,* 2011), and others that show no effect of lairage time on colour (Ekiz *et al.,* 2012). However, yellowness is generally not associated with consumer preference of lamb and is therefore unlikely to have an effect on consumer acceptability (Khliji *et al.*, 2010). Surprisingly, many lambs from both sources and across curfew times failed to reach acceptable levels for *L** (lightness), which consumers associate with quality and freshness, but 100% of lambs achieved acceptable redness. Further analysis combining L and pH>6 is required.

Overall, our results showed little evidence to suggest that extending time in lairage up to 90h affects the objective measures of lamb eating quality. While there were some indications that meat eating quality may be affected (e.g. changes in colour, glycogen and pHu), there was no agreement between the results and the most indicative objective measure of eating quality – shear force – showed no difference between source and time in lairage. Further, all pHu LL measures were under the threshold of 5.8 for eating quality and shelf life across source and time in lairage.

Welfare indicators

All lambs from KI experienced at least 30h water deprivation, while lambs from the Near region varied between 16.25 and 26.2h water deprivation. Interestingly, plasma osmolality measured in this study was consistently higher than baseline osmolality reported in other studies which ranges from 285-300mOsm/L (Pearce *et al.*, 2008; Leake *et al.*, 1984), while other measures of dehydration in this study, such as USG, cooking loss and muscle dry matter were consistently lower than reported benchmarks. Further, Parrott et al. (1996) reported that sheep without access to water were able to maintain plasma osmolality at normal concentrations for up to 48h without access to water, which is significantly longer than what sheep were deprived of water for in the current study (Table 3). Elevated plasma osmolality can cause active conservation of water via the release of an antidiuretic hormone, which would cause USG to remain static (Jacob *et al.* 2006a), as seen in the current study. However, high plasma osmolality also causes water to move out of muscle tissue, which was not the case in the muscle dry matter or cooking loss results. It may be possible that the high plasma osmolality levels were caused by stress rather than dehydration, and this is partially supported by the drop in osmolality as lairage time increases, and also by the higher levels in lambs from Near properties (short travel time) compared to those from KI (Fisher *et al.*, 2010). Nevertheless, plasma osmolality significantly decreased in lambs from both regions, with increasing time in lairage.

Average USG was unaffected by source as well as time in lairage; others have shown a consistent, slow decrease from un-loading to 48h for USG to return to pre-transport levels (Knowles *et al.*, 1996) However, the average USG reported in the current study is lower than other post-transport levels and more similar to base-line levels reported in other studies (Pearce et al., 2008, Jacob *et al.*, 2006b), which indicates a good level of hydration among lambs in the current study. It has been hypothesised that lambs that arrive in lairage hydrated have a static or slightly increasing USG, while those that are dehydrated on arrival are more likely to decrease in USG with time in lairage. Temperature may have also played a role in the relative drop in USG in the current study when compared to others (Pearce *et al.*, 2008), however there was no effect of week on USG results (Appendix).

There were significantly more lambs from KI that were classified as dehydrated (USG > 1.045), with no change with increasing time in lairage, when compared to those from Near properties (Table 11). USG > 1.045 is generally considered to be the point at which tissue hydration begins to be affected, however, there was no difference in muscle dry matter between any of the treatments (Table 15), while Near lambs within MSA curfew limits had a significantly higher cooking loss from both the loin and topside compared to all other groups, indicating a greater level of tissue water holding capacity – which is in direct contrast to plasma osmolality results. The level of dehydration in the current study (28% and 7-12% of lambs from KI and Near properties respectively) is still well below the 43 – 60% observed in a measure of 219 commercial consignments in Victoria and WA, which were measured across a range of seasons, ages and breeds.

The ambiguity between methods for assessing hydration in the current study suggests that one method alone should not be used as a definitive measure of hydration. Despite the higher incidence of lambs from KI being classified as dehydrated, when taken together, our results show limited effect of increasing curfew length on the indicators of animal welfare that were measured in this study. This result is in line with others that have analysed behavioural changes, blood metabolites and other physiological changes associated with transport and curfew times. Extended transport times (up to 48h) and the associated feed and water withdrawal do not, in themselves, pose major welfare challenges to healthy sheep (Fisher et al., 2010). Sheep can recover from slight dehydration within 24h with access to water, and shorter journeys may indeed be more stressful for the animal as no ‘acclimatisation’ period is experienced between loading, the beginning of the journey and un-loading (de la Fuente *et al.*, 2012). Further, the decrease in plasma osmolality, and lack of change in USG with increasing time in lairage in the current study indicates that sheep successfully accessed water in lairage.

Lambs from Near properties had more glycogen than lambs from KI regardless of time in lairage. Glycogen levels are determined by nutrition and pre slaughter stress. Therefore, the difference in glycogen between lamb source may be due to on-farm nutrition or pre-slaughter stress. It is unlikely that there would be consistent nutrition differences between Near farms and the KI farms, so it is hypothesised that the extended travel time or mode of travel is likely to have increased glycogen depletion in the KI lambs and could be an indicator of increased stress in the KI lambs. This translated through to an increase in the number of dark cutting carcases from the KI lambs. Across the 3 muscles measured, there were indications that the KI lambs were exposed to more stress than the Near lambs, regardless of curfew period. Nevertheless, the glycogen levels were at least 17% higher than that required to negatively affect eating quality, suggesting the KI lambs had enough muscle glycogen to buffer against the stress imposed by the increased water or travel time.

Conclusion

It is possible that feed curfew period can be extended up to 72h (and even to 90h) as an alternative pathway for the supply of lamb to MSA specifications. This would align with the LDT trial with the recommendations of slaughter within 48h of dispatch and a minimum of 12h in lairage. It is important to ensure that lambs have access to water to prevent dehydration whilst in lairage. For lambs with extended or complicated transport, such as road and ferry, it will be necessary to ensure that lambs have excellent on-farm nutrition to ensure adequate muscle glycogen to buffer against the stress imposed by extended transport, and decrease the risk of dark cutting meat.

While some of the welfare indicators in this study were contradictory to one another, overall there seemed to be little detrimental effect of increasing feed curfew time to a maximum of 90h on the indicators of welfare measured in the current study. The fact that all sheep across curfew time and source had USG levels lower than baseline levels in other studies suggests their hydration was not affected. Likewise, there was no effect on the most significant objective measures of eating quality. These results reflect others that have analysed behavioural and physiological changes, and subjective measures of eating quality with increasing time in lairage, and found there to be no disadvantage in extending time in lairage. Indeed, time in lairage is beneficial to lambs in recovering from the stress of transport and unloading in a novel environment.

Appendix

Weather during slaughter period

In both Mt. Barker (24km from Lobethal T & R Pastoral) and Parndana, KI the coldest temperatures were experienced in week 1, and the warmest temperatures were recorded in week 2 (Appendix Table 17). Week 2 also had the most rain (5mm total), with thunder and lightning at Lobethal, which caused power failures on Monday night and Tuesday afternoon.

There were no trends between the temperature and plasma osmolality or USG (Figure 3).

Table 17. Weather recorded at Mt. Barker weather station over the trial period.

	Day	Min. temp. (°C)	Max. temp. (°C)	Rain (mm)	RH 09:00	RH 15:00	Wind speed 09:00	Wind speed 15:00
Week 1	Sun	8.1	17.2	0	67	n.r.	15	n.r.
	Mon	6.0	21.1	0	66	27	19	7
	Tues	10.8	27.0	0	27	20	33	30
	Wed	16.8	27.0	0	40	43	7	15
Week 2	Sun	12.0	32.8	0	33	19	11	6
	Mon	21.2	27.1	0.4	71	48	19	33
	Tues	12.2	20.7	4.6	80	55	7	4
	Wed	9.0	21.3	0	61	51	6	15
Week 3	Sun	8.4	21.3	0.4	65	55	4	15
	Mon	7.0	18.4	0	77	60	7	15
	Tues	9.0	17.6	1.4	70	42	11	15
	Wed	9.4	19.0	0	76	57	4	4

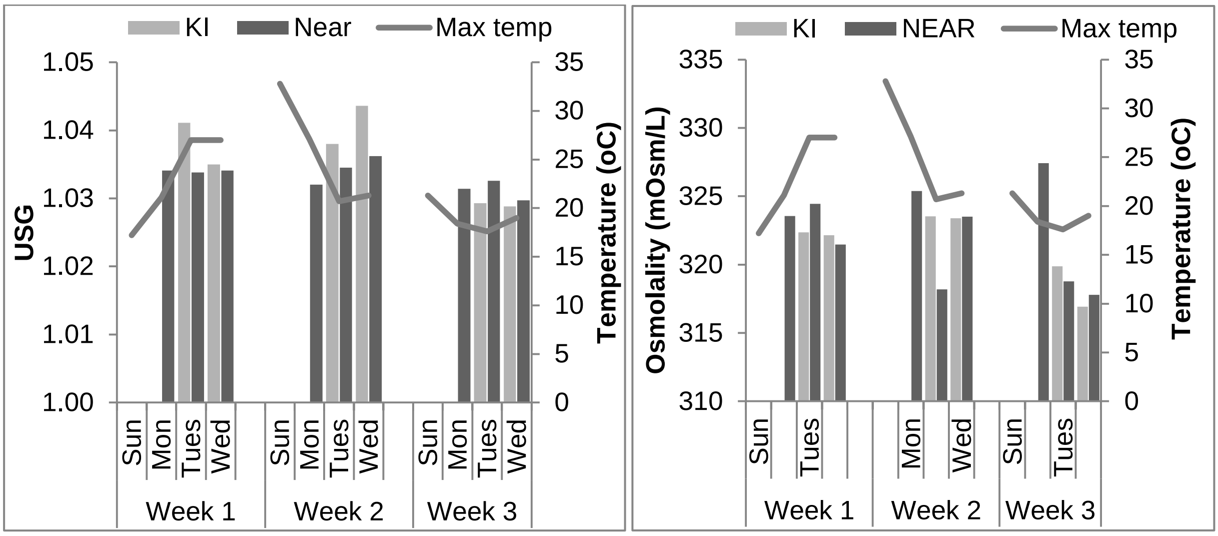


Figure 3. Maximum daily temperature and average (a) urine specific gravity and (b) plasma osmolality.

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